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CITATION:

SHIMIZU, TOMOHITO ...[et al]. Successful spontaneous nesting of the hawksbill turtle (*Eretmochelys imbricate*) at Yaeyama Station, National Center for Stock Enhancement, Japan. Proceedings of the 2nd International symposium on SEASTAR2000 and Asian Bio-logging Science (The 6th SEASTAR2000 Workshop) 2005: 69-74

ISSUE DATE:

2005

URL:

<http://hdl.handle.net/2433/44087>

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Successful spontaneous nesting of the hawksbill turtle (*Eretmochelys imbricata*) at Yaeyama Station, National Center for Stock Enhancement, Japan

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ABSTRACT

With a view to the conservation of the natural stock of the hawksbill turtle (*Eretmochelys imbricata*), we carried out studies on the propagation techniques of this species. This paper deals with the spontaneous nesting of this species under rearing conditions. Adult turtles that were captured off Ishigaki Island between 1999 and 2002 were brought to the center by licensed fishermen. One adult male and three mature females were selected from 29 turtles and they were stocked in a 250kL tank that was connected to a sandy beach for nesting. The females were examined by ultrasonography throughout experimental period. The mean diameter of the follicles ranged from 1.4 - 1.7 cm from January to April; and gradually increased to 3.0 cm from which shelled eggs were laid. The shelled eggs were first observed on 2nd June 2004, but were not observed after September. In contrast, non nesting individuals had not possessed shelled eggs during the experimental period. These results indicate that the formation of shelled eggs is an indicator of nesting. The first nesting was observed on 7th June, with a total of 7 nestlings observed until September. A total of 894 eggs were obtained from two females.

Keywords: hawksbill turtle, spontaneous nesting, rearing conditions

INTRODUCTION

We studied the nesting of the hawksbill turtle *Eretmochelys imbricata* in captivity at the Yaeyama Station of the National Center for Stock Enhancement, Japan, in order to develop a stock enhancement technology. Development of this technology is needed because sea turtle stocks have been damaged by impacts related to human activity, such as beach erosion (Eckert 1990), marine pollution (Hutchinson and Simmonds 1992), egg poaching (Sutanto *et al.* 1989, Mortimer *et al.* 1993), and incidental capture by fishing gear (Abe *et al.* 2003). All sea turtle species are listed in the Convention on International Trade in Endangered Species of Wild Flora and Fauna (Suganuma *et al.* 1999). In particular, the hawksbill turtle has been so highly prized for its tortoiseshell (Kamezaki 1988) that its stocks have become seriously depleted.

Technologies for successful nesting in captivity are very important for enhancing natural stocks without having adverse impacts on these stocks. Although a successful culture method that includes breeding under captive conditions is already established for the green sea turtle *Chelonia mydas* (Ulrich and Parkes 1978, Wood and Wood 1980), there had been no successful cases of nesting by the

hawksbill turtle in captivity until we managed to achieve success in 2004. There have been several previous reports in relation to conservation programs (Witzll 1974) and egg conservation (Wood 1986, Phairot and Sayan 1987).

MATERIALS AND METHODS

Procurement of hawksbill turtles

A total of 37 turtles caught by licensed fishermen in the waters off Ishigaki Island (Fig. 1) between 1999 and 2002 were transferred to the Yaeyama Station of the National Center for Stock Enhancement, Japan. The turtles were reared in a tank with a volume of 400 kL or 60 kL. The water temperature in the rearing tank was not controlled, and seven times of water circulation was made daily in the rearing tank. The turtles were fed anchovy (*Engraulis japonicus*) and squid (*Illex argentinus*) five times a week. Vitamins and calcium powder were dusted onto the feed to supply additional nutrients. Twenty nine turtles (14 male and 15 female) survived until the end of November 2003. Eight turtles died accidentally either from injury at capturing or drowned in rearing tank (was sucked into a drainage hole).

Selection of mature hawksbill turtles

Mature turtles were selected from the group of 29. Males were selected as mature by their morphological features (Owens 1997) and females by the presence of follicles on ultrasonographic testing. Consequently, one male and three females were used for the experiment. Because microchip tags had been implanted previously into the turtles' bodies at the time of procurement, we could easily identify the three females, A, B, and C (Fig. 2). This checking also revealed that females A, B, and C had entered the Station in April 1999, January 2002, and April 2002, respectively. When they entered the station, female A had a SCL 68.4cm and a body weight 30.8kg, female B had a SCL 78.5cm and a body weight 52.7kg, and female C had a SCL 63.0cm and a body weight 26.2kg.

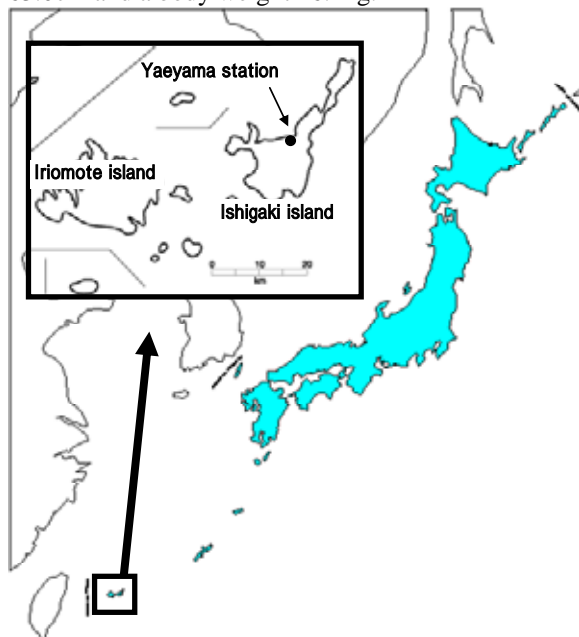


Fig.1 The location of Yaeyama station



Fig.2. Selected matured male and females for the experiment.

Ultrasonographic testing

Ultrasonographic testing (model: Sonosite 180, SonoSite inc.) was used to select mature females.

First the females were placed individually into a water bath filled with tap water. The probe (model: C60, SonoSite inc.) of the diagnostic imaging apparatus was placed on the groin area (Fig. 3). The gonad shape was displayed by monochrome images (Fig. 4). If follicles were recognized in the tested female, we considered her mature. The diameter of the follicle was measured on the display (Fig. 4). Ultrasonographic testing was made once or twice a month between June and September 2004.



Fig.3. Ultrasonographic test.



Fig.4. The ultrasonogram of turtle's follicles.
Arrow shows a diameter of follicle.

Facility

The nesting survey was conducted between June and December 2004. The three mature females and the male were placed in a 200kL tank from January to May 2004. They were then moved to an experimental facility designed for nesting. The facility consisted of a 250kL rearing tank with a recirculation system. The tank was connected to a beach 13 m long and 4.7 m wide, with sand to a depth of 1 m (Fig. 5, 6). The water temperature in the rearing tank was not controlled, but the water was circulated seven times daily. The turtles were fed anchovy (*Engraulis japonicus*) and squid (*Illex argentinus*) at a feeding rate of 2% in body weight daily and they were fed five times a week. Vitamins (ready-made multi vitamin) and calcium powder were dusted onto the feed to supply additional nutrients.

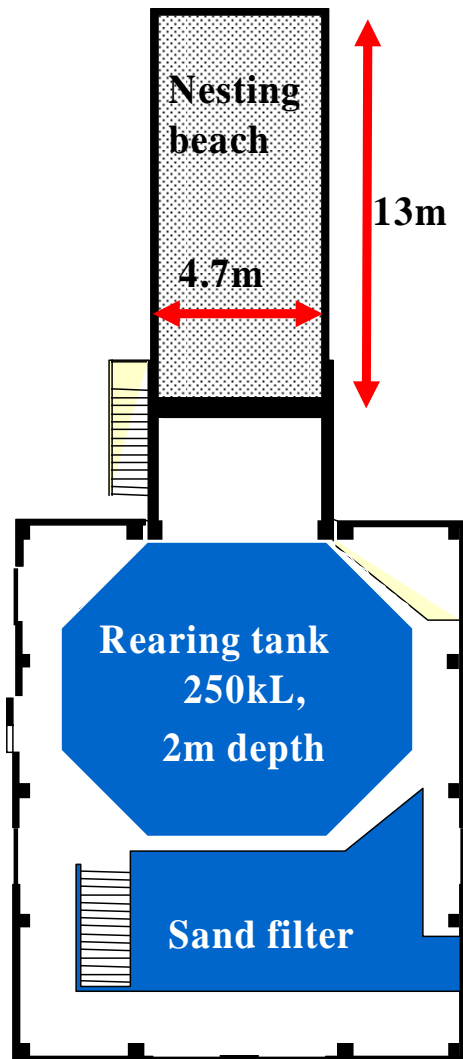


Fig.5. The overview of facility for nesting.

Treatment of eggs

When nesting was observed, the eggs were dug out of the sand with care and their diameters were measured with a caliper. Then the eggs were carefully transferred into an incubator controlled at 29 °C and a humidity of above 90%. It is well known that the sex ratio of turtle offspring is determined not by genetics but by the temperature at which the eggs are incubated (Godfrey *et al.* 1999). Our choice of incubation temperature was determined from a report estimating that the temperature at which 50% of each sex was produced was 29.2 °C (Mrosovsky *et al.* 1992). When the eggs hatched we recorded the hatching day and number of hatchlings.



Fig.6. Nesting beach in the facility for nesting.

RESULT

Relationship between nesting and changes in follicle size

Periodic surveys by ultrasonographic test showed the changes in follicle diameter in the three selected mature females (Fig. 2). The diameters of the follicles in female A fluctuated within the range of 1.4 to 1.7 cm during January to April 2004. After May, the mean diameter increased gradually to 3.0 cm and the eggs began to develop to the shelled stage (above 3.0cm diameter). On 7th June, female A nested for the first time in this experiment. After she had nested, shelled eggs were observed on the scans to the end of September. In October no shelled eggs were visible. Shelled eggs were seen by ultrasonography in female B for the first time in July. Female B first nested on 21 July. After she had nested a temporal decrease in follicle diameter was observed, and then the diameter increased to 3.0 cm again. A few days later we observed the shelled egg, and she nested again on 12 August. Female B stopped nesting in September, and her follicle diameter then gradually decreased. Female C had no shelled eggs and did not show nesting behavior.

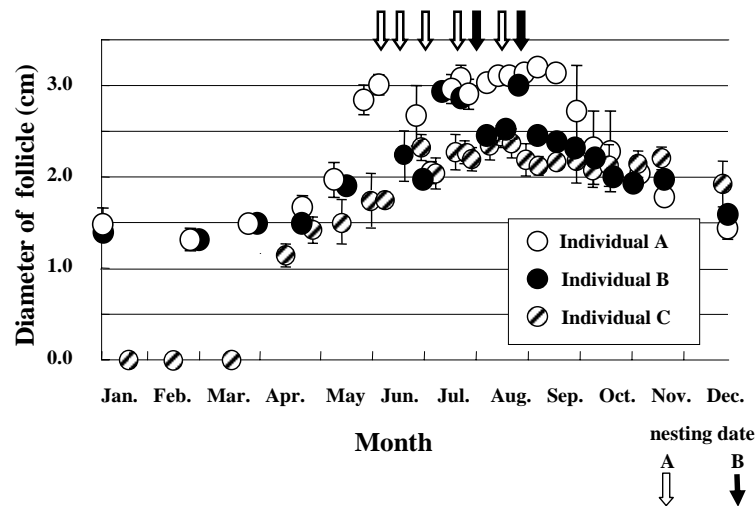


Fig.7. The seasonal variation of follicle diameter on hawksbill turtle during experiment.

Nesting and eggs

A total of seven nestings were recognized in the two females. The total number of eggs obtained from the two females was 894. The mean egg diameter was 3.6 cm (Table 1). After

incubation of the eggs at a temperature of 29 °C for 56 to 59 days after nesting we obtained 309 hatchlings. The total of hatching rate was 34.6% (Table 2).

Table 1. The result of nesting under the rearing in Yaeyama station 2004.

Female	Nesting	Date	Number of eggs	Diameter of eggs
			(ind./clutch)	(average \pm SD, cm)
A	1	7 June	103	3.5 \pm 1.2
	2	14 June	141	-
	3	25 June	159	3.5 \pm 0.9
	4	8 July	160	3.5 \pm 0.8
	5	26 July	*unknown	-
Subtotal			563	
B	1	21 July	153	3.4 \pm 0.8
	2	12 August	178	3.4 \pm 0.9
Subtotal			331	
Total			894	

*: Female A lied eggs in the water in the rearing tank.

Table 2. The hatching result of newly hatchlings in Yaeyama station 2004.

Female	Nesting	Hatching date	Number of hatchlings	Hatching success (%)	Body size of hatchlings		Incubation days (day)
					SCL (Ave. \pm SD, cm)	BW (Ave. \pm SD, g)	
A	1	3 August	20	19.4	3.8 \pm 1.7	12.2 \pm 0.9	57
	2*	19 August	83	98.8	3.9 \pm 1.2	13.2 \pm 0.7	-
	3	22 August	6	3.8	3.7 \pm 1.1	12.5 \pm 0.5	58
	4	5 September	59	36.9	3.6 \pm 1.4	11.5 \pm 0.8	59
Subtotal			168				
B	1	15 September	141	92.3	3.9 \pm 1.6	13.4 \pm 0.8	56
	2		0	0			
Subtotal			141				57.5**
Total			309	34.6**	3.8**	12.6**	

* The eggs were incubated in the nesting beach just before hatching, and 57 eggs were broken at the finding

** Average

DISCUSSION

We observed nesting under artificial conditions from June to August on Ishigaki Island. This nesting period was the same as the wild nesting period reported by Kamezaki (1988) in the same district. Moreover, comparison of the sizes of eggs obtained in this study with the sizes of wild nested eggs, as reported by Kamezaki (1994), showed similar sizes. These results imply that the nesting season in our facility was almost the same as that under natural conditions. However, the egg-hatching rate in this study was 34.6%, which is lower than the 51% to 89.2% reported under natural conditions (Raj 1976, Phairot and Sayan 1987). The reason for this low hatch rate is not clear.

We examined the new information revealed by ultrasonographic testing about the process of egg maturation. The changes in follicle diameter revealed that all of the shelled eggs in mature females were laid during the one nesting, and that the remaining follicles without shells were absorbed into the body after the nesting season. Presumably the formation of shelled eggs was a sign of nesting. Further studies of the relationship between the appearance of shelled eggs and nesting may enable us to determine whether the female is going to nest and to estimate the prospective nesting day. We found that the ultrasonographic test adopted here was effective for non-invasively monitoring the condition of mature females.

Only three mature females could be examined. The reason for the low ratio (23%) of mature females might have been that many of the females introduced in our station, were smaller than the minimum size (66 cm, mean straight carapace length) of mature females as reported by Witzell (1985). If we can accelerate growth, then our technique for turtles to nest will be more practicable in captive turtle rearing. It has been reported that the growth rate of hawksbill turtles under natural conditions (Kamezaki 1987) is greatly inferior to that under culture conditions (Sukwong and Kao-Eian 1994). It has also been determined that the stomach of the wild hawksbill turtle contains not only animal matter but also vegetable matter. Wild hawksbill turtles have revealed large number of sponges spicules in the gut, because sponges that are important diets in hawksbill turtles. (Leon and Bjorndal 2002). The reason for the growth difference may be explained by the hypothesis that the feed used for rearing has more calories than that obtained under natural conditions. We have thus advanced the merits of rearing prospective broodstock, because we can control the content and quantity of the feed used.

We now have a few measures that can be used to counter the decline in our sea turtle resources. Ongoing features of the conservation program are the improvement of fishing gear against bycatch

(Abe *et al.* 2003) and egg conservation (Wood 1986, Phairot and Sayan 1987). In comparison with these conservation activities, stock enhancement can be a more affirmative and planned measure because it opens up the possibility of maturation, nesting, rearing, and releasing techniques. The technology of nesting in captivity is thus a very important component of stock enhancement.

ACKNOWLEDGMENTS

We would like to thank the staff of the Yaeyama Station of the Fisheries Research Agency, Japan, for their support. We give grateful thanks to Keiichi Mushiake, Masakazu Oka and Yoichi Takahashi for their valuable comments on this manuscript.

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